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Original Article

Copper, selenium, zinc, and iron deficiencies in male athletes

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Abstract:
Trace elements play a significant role in biological processes which are of great interest to athletes, coaches, and sports professionals. Iron, copper, zinc, and selenium act also as antioxidants, participating in the catalytic action or in stabilizing the active centre of antioxidant enzymes catalase, Cu,Zn-superoxide dismutase and glutathione peroxidase. The aim of this study was to investigate the deficiency of the trace elements iron, copper, zinc, and selenium in athletes from different sports, in addition to assessing their relationship with the activity of antioxidant enzymes in which they participate. Venous blood was taken from 43 male athletes with a mean age of 21.0±4.10 years (6 karatekas, 18 soccer players, and 19 wrestlers) and complete blood count, serum concentration of microelements (Fe, Cu, Se, and Zn) and ferritin, and activities of Cu,Zn-superoxide dismutase and glutathione peroxidase in packet cell volume were measured. The results showed that iron deficiency, without anemia, was the most frequently established microelements' deficiency, and ferritin was more sensitive predictor of iron deficiency than serum iron concentration. Athletes with chronic inflammatory diseases are vulnerable to iron deficiency. Statistically significant lower values of ferritin, copper, and superoxide dismutase activity were established only in the sample of wrestlers. In conclusion, athletes with heavy training regimen (2 workouts per day plus strength training), such as the wrestlers in this study, are more vulnerable to microelements deficiency. Further studies are needed to show what serum concentrations of copper, zinc, and selenium provide the highest activities of the antioxidant enzymes superoxide dismutase and glutathione peroxidase.

Key Words: **microelements, ferritin, antioxidant enzymes, Shotokan karate, soccer, wrestling**

Introduction

Trace elements play a significant role in biological processes, such as energy supply, oxygen transport, heart rate regulation, bone metabolism, inflammatory response, immune protection, and others that are of great interest to athletes, coaches, and sports professionals (Kerksick et al., 2018; Kreider et al., 2010; Misner, 2006; Speich et al., 2001; Williams, 2005). Some trace elements e.g. iron, copper, zinc, selenium, are also crucial as antioxidants, as they participate in the catalytic action or in stabilizing the active centre of antioxidant enzymes. For example, copper and zinc ions are responsible for the antioxidant enzyme superoxide dismutase activity, with Cu^{2+} participating in the catalytic properties of the enzyme, while Zn^{2+} stabilizes the molecule. Catalase is an antioxidant iron-containing enzyme. Five out of the eight identified isoforms of glutathione peroxidase contain the trace element selenium (Se) (Morón & Castilla-Cortázar, 2012). It has been suggested that the activity of these enzymes may serve as one of the indicators for the body's supply of the microelements copper, zinc, and selenium (Institute of Medicine (US) Panel on Dietary Antioxidants and Related, 2000; Institute of Medicine (US) Panel on Micronutrients, 2001). Antioxidant enzymes maintain the pro/antioxidant balance in the body under physiological conditions, neutralizing reactive oxygen species (ROS), which due to their high reactivity can oxidize the biomolecules and lead to structural and functional changes in cellular organelles, tissues, and organs. Several factors can disturb the pro/antioxidant balance and cause oxidative stress (OS)(Halliwell & Gutteridge, 2015) including smoking (Beschasnyi et al., 2021), alcohol consumption (Stefanyk & Mulyk, 2019), high-intensity and prolonged physical activities (Raiola et al., 2016). Thus, the oxygen consumption increases between 10- and 15-times during exercise, which leads to increased generation of reactive oxygen species (ROS). Other sources of ROS in physical activity are catecholamines, prostanoid metabolism, xanthine oxidase and NAD(P)H oxidase activities, phospholipase A2, lipoxygenases, and several secondary sources such as macrophages and neutrophils via γ-β-interferon IF interleukin-1 (IL-1) and tumor necrosis factor (TNF) (Jackson et al., 2002; Peake & Suzuki, 2004). Oxidative stress can cause muscle damage, fatigue, and poor performance (Baltaci et al., 2016; Powers et al., 2020). Therefore, the lack of micronutrients in the body

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can lead to increased oxidative stress and reduced level of fitness, and as a consequence worsen sports performance. Micronutrient deficiencies are often observed in the general human population. The results of population-based studies showed that the intake of micronutrients did not reach the RDA, and the data for selenium, iron, and zinc are particularly worrying due to the reported significant deficits of 60%, 30%, and 17% of the RDA, respectively (Bailey et al., 2015; Kumssa et al., 2015). In athletes, micronutrient deficiencies are also common, and they are associated with diet, loss of sweat and urine (Anderson & Guttman, 1988; Micheletti et al., 2001). In runners who did not take supplements, dietary intake of zinc, iron, and copper was estimated to be only 75%, 50%, and 8% of the recommended daily intake, respectively (RDA) (Clarkson, 1991). Research has showed that micronutrient deficiencies negatively affect athletes' physical condition and performance (Heffernan et al., 2019). Therefore, the addition of micronutrients can enhance their natural effect in the body and thus improve tolerance to heavy physical exertion, reduce recovery time after competition or training, and positively affect athletes' health.

Considering that microelements are an essential factor for overall physical health and sports performance, including as a part of antioxidant protection of cells, the purpose of the present study was to investigate the deficiency frequency of the trace elements iron, copper, zinc, and selenium in athletes from different sports, and to assess the relationship of these elements with the activity of antioxidant enzymes in which they participate.

Material & methods

Participants

This study included 43 male athletes with a mean age of 21.0±4.10 years. Six of the participants practiced Shotokan karate, 18 were soccer players, and 19 were wrestlers. All participants declared that they had not taken any supplements during the last 12 months. All athletes signed informed consent prior to the study, and the procedures involving human participants were in accordance with the 1964 Helsinki declaration and the national research committee's ethical standards.

Anthropometry

The height of the athletes was measured by a stadiometer with an accuracy of 0.5 cm. The body weight and body composition were assessed using InBody 230 (InBody Co., Ltd., Korea). The following parameters were determined: body mass index (BMI), relative body fat (BFR%), and percentage of skeletal muscle mass (SMM).

Blood collection and assays

Venous blood was taken from the participants by a medical professional in two vacutainers. The blood from the first vacutainer was used for measurement of complete blood count by hemoanalyser (Alinity HQ, Abbott Diagnostics, USA) and the concentration of microelements (Fe, Cu, Se, and Zn) in the serum by atomic absorption spectrometer (PinAAcle 900Z, PerkinElmer, USA). A licensed laboratory performed both measurements. The norms given by the device manufacturers were accepted for normal upper and lower limits of both the hematological parameters and the serum concentrations of the microelements. In blood, ferritin serum concentration was measured by Ferritin Enzyme Immunoassay Test Kit (BioCheck, Inc, USA) Cat.# BC-1025. The blood from the second vacutainer was used for biochemical analyzes. The blood was centrifuged at 3000 rpm, and plasma was discarded. Blood cells were washed twice with saline, and the resulted packet cell volume (PCV) was stored at -20°C until biochemical analysis. Just before analysis, the PCV was diluted in phosphate-buffered saline (pH 7.4) to obtain 5%PCV. The hemoglobin (Hb) concentration in 5%PCV was measured by reagents purchased by Teco Diagnostics (USA). The erythrocyte Se-dependent glutathione peroxidase (GPx) activity was measured in 5%PCV by Glutathione Peroxidase Cellular Activity Assay Kit (Cat.# CGP1, Sigma-Aldrich Ltd, USA). The erythrocyte superoxide dismutase (SOD) activity was measured after hemoglobin precipitation with a mixture of chloroform: ethanol (3:5) (Maral et al., 1977) by SOD Determination Kit (Cat.# 19160, Sigma-Aldrich Ltd, USA). The SOD activity was expressed as a percentage of formazan production inhibition. Z-scores based on the data average value and standard deviation from this study were calculated because of the lack of established norms of erythrocyte SOD and GPx activities.

Statistical analysis

The obtained data was calculated by variational analysis. The Shapiro-Wilk test of normality was used, and the differences in mean values between groups were calculated by ANOVA or Kruskal-Wallis analysis using Prism 7.04 (GraphPad, Software, Inc).

Results

The mean values of the anthropometric data of the participants are presented in Table 1. According to WHO (WHO, 2021), among all participants in this study, only one athlete (S10) had low BMI (BMI=18.3 kg/m^2); 15 athletes had BMI>25 kg/m^2 (overweight) as 13 of them were wrestlers, and 2 were karate players. Two athletes (karateka S5 and wrestler S42) had BMI slightly above 30 (obesity). The athletes with a BMI> 25.0 were identified as overweight according to WHO standards (WHO, 2021). However, all these 15 athletes had BFR% under 20.0% and high percentage muscle mass with a mean SMM of 48.6 ± 2.14%. The two athletes with

BMI> 30.0 were accurately assessed as obese according to the norms of WHO (WHO, 2021) because they had high BFR> 20.0% and low muscle mass of around 40.0%.

Table 1 Anthropometric data of the athletes (n=43)

	Height	**Weight**	**BMI**	**%BFR**	**SMM**
	[cm]	**[kg]**	**[kg/m2]**	**[%]**	**[%]**
Average	177.3	76.6	24.35	13.0	49.8
SD	7.21	10.57	2.69	4.83	2.76
SE	1.10	1.61	0.41	0.74	0.42
Max	190	101.0	30.6	29.3	53.8
Min	161	55.4	18.3	5.9	40.6

The obtained data from the hematological assessment is presented in Table 2.

Table 2 Hematological data of the athletes (n=43)

	WBC	**N**	**Eo**	**LYM**	**Mo**	**Ba**	**RBC**	**Hb**	**HCT**	**MCV**	**MCHC**	**MCH**	**RDW**	**PLT**
	G/L	**%**	**%**	**%**	**%**	**%**	**T/L**	**g/L**	**L/L**	**fL**	**pg**	**g/L**	**%**	**G/L**
Upper limit	**10.5**	**73.7**	**7.3**	**48.30**	**12.7**	**1.7**	**5.40**	**160**	**0.44**	**98**	**32.0**	**360**	**14.5**	**440**
Lower limit	**3.5**	**39.3**	**0.6**	**18**	**4.4**	**0.00**	**3.70**	**115**	**0.35**	**82**	**26.5**	**295**	**11.5**	**130**
Mean	6.64	52.6	2.07	35.7	9.15	0.44	5.08	152.2	0.46	91.60	30.07	328.3	13.1	246
SD	1.77	8.38	1.82	7.89	1.88	0.35	0.43	8.83	0.03	5.79	1.96	6.329	0.96	54.97
SE	0.27	1.28	0.28	1.2	0.29	0.05	0.07	1.34	0	0.88	0.30	0.97	0.15	8.383
Max	13.8	78.5	9.6	47.6	13.5	1.3	6.7	179.0	0.6	100.0	32.9	342.0	15.3	414.0
Min	4.0	40.2	0.3	7.7	5.8	0.0	4.3	136.0	0.4	63.0	20.4	317.0	10.0	134.0

The mean hematological parameters of the athletes were within the normal range accepted by the laboratory conducting the analyses. Deviations beyond accepted norms in hematological parameters were observed only in two individual athletes: 1) S28 (wrestling) with MCV = 63 fL below lower limit; MCHC = 20 pg; and 2) S11 (soccer) with WBC 13.8 G/L above upper limit, N% = 78.5%; N# 10.8 G/L above upper limit; LYM% = 7.74 below lower limit; L# 1.07 G/L below lower limit. The latter had exacerbated chronic tonsillitis. According to WHO criteria, no athlete had evidence of anemia (Hb less than 130 g/L, norms for men) (WHO, 2011a).

Almost all mean and individual serum concentrations of the assessed microelements were within the accepted normal range (Table 3) with only three exceptions: 1) one karateka (S3) had a reduced serum concentration of selenium - 386 nmol/L; 2) one soccer player (S11) had a reduced serum concentration of iron - 8.5 µmol/L; and 3) one wrestler (S43) had an increased serum concentration of copper - 32.3 µmol/L.

Table 3 Serum copper, selenium, zinc, and iron of the athletes (n=43)

	Cu	**Se**	**Zn**	**Fe**
	µmol/L	**nmol/L**	**µmol/L**	**µmol/L**
Upper limit	**24.3**	**1123**	**24**	**28**
Lower limit	**13.2**	**423**	**12**	**10**
Mean	18.3	762.88	17.69	20.43
SD	2.06	128.64	2.04	5.75
SE	0.31	19.62	0.31	0.88
Max	22.6	1024.0	23.3	32.3
Min	14.2	386.0	14.2	8.5

Among the 43 athletes, 15 showed ferritin values below the lower limit of 15.0 ng/ml (WHO, 2011b). None of the athletes had iron overload; the measured serum concentration of ferritin was very below the upper limit of 200 ng/ml (WHO, 2011b) (Table 4).

Table 4 Serum ferritin concentration, erythrocytes SOD, and GPx activity of the athletes (n=43).

	Ferritin	**SOD**	**GPx**
	ng/ml	**%Inhibition**	**U/g Hb**
Upper limit	**200**	**NA**	**NA**
Lower limit	**15**	**NA**	**NA**
Mean	27.89	51.5%	35.87
SD	24.35	5.34%	7.92
SE	3.71	0.81%	1.21
Max	92.2	59.6%	58.4
Min	0.1	41.6%	20.3

NA – Not available

The calculated mean activity of erythrocyte SOD was 51.5±5.34% inhibition of superoxide anion radicals ($O_2^{\bullet -}$) generation. Four athletes showed SOD activity Z-score<-1.0 (1 soccer player and 3 wrestlers). The lowest Z-score was =-1.85.

The mean erythrocyte GPx activity was 35.87 ± 7.92 U/g Hb. Five athletes showed GPx activity Z-score<-1.0 (2 soccer players and 3 wrestlers). The lowest Z-score was -2.10.

Table 5 compares the markers related to the microelements' status between wrestlers, karatekas, and soccer players. Statistically significant lower values of ferritin, copper, and SOD activity were established only in the sample of wrestlers.

Table 5 Comparison of microelements status-related markers between the wrestlers, karateka, and soccer players.

	Wrestlers	**Karatekas**	**Soccer Players**
Hb [g/L]	151.7±10.92	154.7±6.743	151.9±7.112
Ferritin [ng/ml]	14.96±9.54	43.35±25.28 *	36.39±28.86 *
Fe [µmol/L]	20.44±5.682	21.27±6.545	20.14±5.864
Se [nmol/L]	714.3±102.5	818.5±236.9	795.6±91.59
GPx [U/g Hb]	32.82±7.167	40.32±6.376	37.59±8.247
Cu [µmol/L]	17.27±1.445	19.03±2.484	19.15±2.079 *
Zn [µmol/L]	17.27±1.977	16.58±1.42	18.5±2.053
SOD [%inhibition]	47.42±2.378	54.02±4.847 *	54.93±4.944 ***

*- p<0.05 v.s. wrestlers, ***- p<0.001 v.s. wrestlers

Discussion

This study aimed to establish the frequency of iron, copper, zinc, and selenium deficiency in athletes from three different sports, since these microelements are essential for maintaining good health, and are also important for high sport performance and achievements. Furthermore, the lack of a certain microelement can negatively affect the body's antioxidant defense against the ROS generation, especially in athletes with high-intensity physical activity.

Iron is essential to every cell, having a fundamental role in oxygen transport, and it is essential for energy production and efficient functioning of all of the organs in the body (Wang & Pantopoulos, 2011). When iron is diminished in the body, a condition named "iron deficiency" is observed, which is most commonly established by low ferritin levels (WHO, 2011b). When iron deficiency continues for a long time, iron deficiency anemia occurs (WHO, 2011a). Iron deficiency, with or without anemia, is widespread among athletes due to increased loss of iron through sweat, loss of hemoglobin in the urine when red blood cells are destroyed because of fever, and blood circulation, or injuries caused by mechanical blows to the surface (Fallon, 2008; Malczewska-Lenczowska et al., 2017; Rowland, 2012; Spodaryk, 2002). Both disorders and particularly anemia, adversely affect physical condition and performance, provoking weakness, general fatigue/exhaustion, heart rate increase and shortness of breath during exercise, headaches, and dizziness (Clénin et al., 2016). Iron supply during a menstrual cycle in women lead to increase of hematocrit, erythrocyte count, hemoglobin and VO_2max (Sinaga et al., 2018). In this study, we did not find athletes with anemia considering the WHO lower limit of hemoglobin set at 130.0 g/L (WHO, 2011a). Only one athlete (S11) had a low serum concentration of iron (8.5

µmol/L) and low serum ferritin (2.6 ng/mL), along with high WBC, N%, N# 10.8 G/L, and low LYM% and L# 1.07 G/L. This athlete had exacerbated chronic tonsillitis. Therefore, it could be suggested that iron deficiency was a consequence of chronic inflammation, which is in line with the literature data (Cappellini et al., 2017). Among the athletes assessed in this study, 15 were with low ferritin values: 5 of them were soccer players (from 18 i.e. 27.7%) and 10 were wrestlers (from 21, i.e. 47.6%). These findings showed an unexpectedly high frequency of iron deficiency in male athletes, especially among the wrestlers. Suboptimal intake of minerals was reported for athletes actively attempting to lose weight to meet the standard weight categories in competitions (Lukaski et al., 1990). All athletes with low serum ferritin had normal serum concentrations of iron except one (S11). This showed the low diagnostic power of serum iron concentration as an iron deficiency index confirming the conclusion that ferritin concentration is reasonably sensitive, and it is a very specific test for iron deficiency in people (Garcia-Casal et al., 2021).

The serum concentrations of copper, zinc, and selenium in all athletes in this study were normal, except in one athlete (S3) who had a lower Se serum concentration. Widespread deficiencies have not been documented for Cu, and Se and there is no established relationship to performance (Clarkson, 1991). Although there are no clinically significant deficiencies of these elements, it is questionable if they are at the optimal level for a good performance. Athletes may have a zinc deficiency induced by poor diet and loss thought sweat and urine. Limited data exist on the relationship of Zn status and performance (Clarkson, 1991).

Our interest in these microelements was mainly related to their role in the body's antioxidant defenses and the assumption that they would affect cellular pro/antioxidant balance at suboptimal concentrations. It is well known that athletes are exposed to a higher level of oxidative stress during physical loading (D'Angelo & Rosa, 2020; Powers et al., 2020). Although the low level of OS triggers adaptive responses, the higher levels induce damages at cellular level, which are further reflected at the physiological level. OS induce muscle fibre damage eventually results in muscle fatigue (D'Angelo & Rosa, 2020), and it is involved in the pathophysiology of overtraining syndrome (Tanskanen et al., 2010). It has been reported up-regulation of SOD, GPx, and catalase activities in skeletal muscle in endurance exercise training (Mankowski et al., 2015; Radak et al., 2013). The wrestlers investigated in this study, in contrast to the karatekas and soccer players, showed lower serum concentrations of copper and a significantly lower Cu,Zn-SOD activity in this group. These results are in accordance with the findings that SOD activity is a good predictor of copper deficiency (Institute of Medicine (US) Panel on Micronutrients, 2001). Although there were no statistically significant differences between groups in our study, lower Se concentration along with lower GPx activity in wrestlers were observed. It was reported that 12% of the total selenium in the plasma was bound to GPx, and changes in the Se concentration of the body directly affect glutathione peroxidase activity (Baltaci et al., 2016). Thus, the reduced activity of antioxidant enzymes is a prerequisite for OS development. However, further studies with a larger number of subjects are needed.

Conclusions

Iron deficiency without anemia is the most frequently established microelements' deficiency in this study. Ferritin is the most sensitive predictor of iron deficiency in male athletes. Athletes with chronic inflammatory diseases should consider the possibility to be assessed as iron deficient. Serum iron concentration has low diagnostic power as an iron deficiency index. Athletes with a heavy training regimen (2 workouts per day plus strength training) and periodically dietary restriction (for pre-competition weight control), such as the wrestlers in this study, are more vulnerable to microelements deficiency. These athletes should consume food rich in micronutrients and take supplements. Research on athletes' microelement supply should be aimed towards establishing not only deficiencies but also determining whether the microelements intake is within optimal limits for the practice of their sport. Further studies are needed to show what serum concentrations of copper, zinc, and selenium provide the highest activities of the antioxidant enzymes SOD and GPx which are depended on these microelements.

Conflicts of interest: The authors declared no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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